

PRELIMINARY REPORT ON THE NUTRITIVE VALUE OF CERTAIN AUSTRALIAN GRASSES.

BY MARGARET H. O'DWYER, B.Sc., Science Research Scholar in the University of Sydney.

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i. *Introduction.*

In this paper it is proposed to embody the results of some research work which has been carried out by myself in the Research Laboratory of the Agricultural Department in the University of Sydney. As yet the work can only be regarded as being in a preliminary stage, but it is hoped later, in further publications of the series, to discuss more fully the economic aspect of the question and to be able thereby to increase the value of the results to pastoralists and agriculturists in this State and in the Commonwealth.

In view of the amount of attention which has been given to this subject in the United States within the last two decades, it is remarkable that, in Australia, which depends so much on its pastures, the value of the native grasses should not have received greater recognition from the "Man on the Land," for experts have often drawn attention to their drought-resisting qualities, and have emphasised the value of their cultivation.

The experience which has been gained in the United States should be invaluable to us. There, also, they have suffered from the results of overstocking (Pammel, 1901), many native grasses having died out from this cause. Great efforts, however, have been made there to renew the worn out pastures by the systematic cultivation of their most valuable indigenous grasses (Lawson-Scribner, 1896; Pammel, 1901; Sampson, 1914). These pastures show continuous improvement from year to year.

Something of the same kind must be attempted in Australia at no very distant date if we are to retain the valuable heritage we possess in our native grasses. A beginning has been made, for most of the State Experiment Farms possess experimental grass plots. The enormous value of these in stimulating interest in the formation of permanent native grass pastures will be evident.

In the examination of American grasses and of feeding stuffs generally, the chemists are working in conjunction with the botanists (Griffiths, 1915; Knight, 1905, 1906, 1908, 1911). The advantages accruing from teamwork of this kind must be apparent to every thinking person, and it is to be hoped more of it may be done in Australia than has been the case so far.

ii. *Material.*

Through the courtesy of the Director of Agriculture, for which I am very grateful, the material has, for the most part, been sent in from the various State Experiment Farms. Any slight abnormalities which may exist as a result of cultivation on the Farms should be more than compensated for by obtaining the material in a pure state, which would be almost impossible if it were taken from the grass paddocks of an ordinary farm or station.

In every case a sample of the grass analysed has been kept, so that its identification can be verified at any time.

Stages of Growth.—On beginning this work it was found advisable, if any systematic research was to be conducted, to obtain the material in certain well-defined stages of growth. Each grass has, therefore, been procured as far as possible in—

(a) what is known as the medium stage, or about half-way between the time when it begins to shoot and the early flowering period. Grasses are often fed off to stock at this stage, which is said to contain the largest amount of moisture and crude protein,—the latter, however, being largely composed of amino acids and other so-called immature proteins (Armsby, 1911; Vipond, 1914), and the smallest amount of crude fibre, the percentage of which appears to increase rapidly as the grass grows older (Pammel, 1901, p. 411).

(b) The second stage chosen for analysis is the early flowering period. For most of the analytical work on grasses which has been done in the United States, this stage and the third stage, mentioned later, have been used (Griffiths, 1915; Knight, 1906).

(c) The third stage being used for analysis is that at which the seed is quite set.

Condition of Material.—Most of the analytical work has been done on the air dried material. In the first place it was found impossible to get material, which had to come any great distance, in a perfectly fresh condition. In one case, which will be referred to later, a grass arrived from Glen Innes in a wilted condition, and on being dried and analysed showed a different protein content from another sample of the same grass in the same stage, which had been dried on the Farm.

The material is weighed at the Farm as soon as cut, dried and then re-weighed, the difference in weight representing the moisture content other than hygroscopic moisture. Unfortunately, none of the Farms, except Hawkesbury Agricultural College, possess even a rough chemical balance, so that inaccuracies will be found in the percentages of total moisture. At present these seem unavoidable. The material must be weighed immediately after being cut, as it begins to lose water at once.

For analytical purposes the material is ground up until it is fine enough to pass through a 1 mm. sieve (Brunnich and Smith, 1907, p. 343). It is then stored in airtight bottles and kept in the dark until required (Knight, 1906, p. 5).

iii. *The Value of Digestibility Experiments.*

In a publication entitled "The Relative Value of Feeding Stuffs—Experiments with the Respiration Calorimeter in Co-operation with the Bureau of Animal Industry" (Armsby, 1907), the writer states that the only safe basis for a comparison of the nutritive value of feeding stuffs is the actual experiment upon the animal, in which the real gain or loss of flesh and fat is accurately determined. In other words, that the only way to ascertain the nutritive effect is to actually determine it. Kellner (1909) found that, in the case of coarse fodders, the actual results were much lower than the computed ones. The difference was found by him to be very closely proportional to the amount of crude fibre present, amounting to 617 calories for each lb. of the total crude fibre. When this deduction was made, the computed results agreed very closely with those found.

Pammel, etc. (1901) give the following digestion coefficients for pasture grass:—

| | Digestion Coefficients. | Digestible Ingredients. |
|-------------------------|----------------------------|----------------------------|
| Dry matter | 69 | 13.8% |
| Organic matter | 70 | 12.6% |
| Crude protein | 66 | 2.3% |
| Crude fibre | 74 | 3.0% |
| N free extract | 73 | 7.1% |
| Ether extract | 55 | 0.4% |

The digestion coefficient is the percentage of the particular nutrient which is digested during the passage of food through the animal.

The percentage of digestible ingredients is found by multiplying the percentage of each ingredient in the substance by its digestion coefficient.

Hence if the percentage of protein in a feeding stuff is 7.4, and the digestion coefficient is .59, the percentage of digestible ingredients in the protein content of the feeding stuff will be $(7.4 \times .59) = 4.4$ (Pammel, 1901).

The figures given in the table may serve to indicate roughly, at any rate, the digestibility of similar feeding stuffs in Australia, but it is to be hoped that before long, digestibility experiments may be carried out in this State, for, without these, no completely accurate basis of the nutritive value of feeding stuffs can be arrived at.

iv. *Discussion of Methods.*

Up to the present the official methods of the Association of American Agricultural Chemists, as revised up to the end of the year 1919, have, in the main, been followed. (Assoen. of Official Agricultural Chemists, 1920.).

The principal reasons for adopting the American methods in this early stage of the work are:—

(a). A large amount of investigation into suggested modifications in methods has been carried out by the American Agricultural Colleges, Bureaux of Chemistry, etc. (Francis, 1916; Bidwell and Walton, 1916; Bidwell, 1916; Francis, 1919; Clarke, 1920; Phelps and Daudt, 1919), and the methods have apparently been subjected to very close scrutiny before being classed as official (Assoen. Official Agricultural Chemists, 1920, Introduction).

(b). Only by practically adopting the same methods, is it possible to make any comparisons between the analyses of our native grasses and those of the United States.

Certain interesting modifications of these methods, however, are of considerable value, and these I hope to make the subject of a further communication in the future. Dr. Petrie (1908), discussing those used for the precipitation of the proteins, gives an account of some preliminary experiments which he carried out with the seeds of *Acacia pyrenantha*, copper hydroxide, (Stutzer's Reagent), tannin salt solution and alcohol being severally used as precipitants. He found that, apparently, alcohol and tannin salt solution gave distinctly more reliable results than copper hydroxide which is the reagent used in the official American methods. These reagents might also be used to advantage in the precipitation of the proteins in grasses. Similarly acetone has been suggested as a precipitant for the proteins (Weyl, 1910).

No attempt has been made so far in this work to examine the non-protein nitrogen in the grasses. Although Van Slyke's method (1911-12) appears at first sight to be suitable for the purpose (Grindley and Eckstein, 1916; Grindley, etc., 1915), other writers (Dowell and Menaul, 1919; Görtner, 1918; Görtner and Holm, 1917; Hart and Sure, 1916) show that furfural and dextrose tend to react on the amino acids and protein hydrolysates (Dowell and Menaul, 1919, p. 131), and that consequently an unusually large percentage of nitrogen is found in the humin. This weakness in the method has also been noted by Grindley, Joseph and Slater (1915). From a consideration of their own results, confirmed as they were by Görtner (1918), Hart and Sure conclude that the method of direct hydrolysis for the estimation of amino acids in feeding stuffs by the Van Slyke method is inapplicable, and that the results so secured will be open to question. It is further suggested by them that, in the present unsatisfactory status of the methods for estimating the amino acids in the complex protein-carbohydrate mixture of feeding stuffs, the only reliable procedure for obtaining an insight into the nutritive worth of the proteins in such a mixture is the biological one.

All the writers mentioned above, however, as well as Nollan (1915), Kellner (1910), and Brunnich and Smith (1907), stress the great importance of the non-protein nitrogenous compounds of feeding stuffs, and especially of the amino acids. In the actual determination of proteins in the grasses, I have followed the Kjeldahl-Gunning-Arnold method recommended by the Association of Official Agricultural Chemists of America (1920), and have found it to work satisfactorily. Slight modifications in the official methods of estimating proteins have been suggested from time to time (Brill and Aseavili, 1917; Phelps and Daudt, 1920), the last named writers suggesting that the use of rubber stoppers in the apparatus, as in the official method, may contribute small amounts of ammonia-reacting substances, and recommending that cork stoppers, entirely covered with tin foil, be used instead. In the determination of true protein, or albuminoid nitrogen, Stutzer's Reagent has been employed as a precipitant for the proteins (Assocn. Offic. Agric. Chemists, 1920).

Crude Fibre.—The acid-alkali method has been used in estimating the crude fibre in the grasses (Assocn. Offic. Agric. Chemists, 1920). Although the method may have many drawbacks (Bidwell and Walton, 1916; Francis, 1916), it is usually considered superior to other methods.

Bidwell and Walton (1916) point out that crude fibre is not the name of a definite substance, but only a convenient and somewhat descriptive term used to denote the result obtained by treating a feed by the official method. "Inasmuch," they say, "as crude fibre designates simply a numerical value obtained by following the official method which is strictly an empirical one, any devia-

tion therefrom will give incorrect results. This is the strongest and all important objection to any material change in a method for official endorsement" (1916, p. 34). These writers have found that, by observing certain precautions, the results of the official method will check very closely, and the difficulties and tediousness will be eliminated. In fact, they have been unable to make the determination by any of the proposed modifications in any less time than by the official method. J. A. and E. W. Voeleker (1918) recommend a modification of the official method involving the use of a 2% solution of the acid and alkali instead of the 1.25% solution used by the American chemists. Brunnich (1907) is of the opinion that König's method (1898) is superior to the acid alkali method, and that the resulting crude fibre is free from pentosans. König's method has, however, been unfavourably criticised by American chemists, on account of its slow filtration and because of the variation in duplicates. Brunnich, however, claims to have overcome these defects (Brunnich and Smith, 1907). In a publication entitled "The Feeding Value of Cereals, as calculated from Chemical Analyses" Chamberlain (1909) states that crude fibre, though only slightly digestible, plays a very important part in digestion. "It acts as a dilutant," he says, "of the more concentrated portions of the food, such as starch; necessitates for the whole food thorough mastication and prevents it from becoming too compact; in other words it keeps the food mass porous and open to the action of digestive fluids. If, however, the crude fibre is in excess, the amount of energy expended by the animal in securing and digesting the food is so great that its ultimate nutritive value is correspondingly diminished."

Ether Extract.—This contains, as well as fat, waxes, chlorophyll and some of the organic acids. It is considered to be very impure in the case of the coarse fodders, containing sometimes as much as one-half of non fatty substances. The method used for this estimation is again the American Official method. Ward (1917, pp. 326—327) recommends that, in order to overcome errors arising from the porosity of corks used in the Soxhlet apparatus and the solubility of certain constituents of the cork in the extraction solvent, the cork should be heated for two hours on a boiling water bath in a solution of gelatin (previously soaked in cold water for five or six hours and then melted) in a quarter of a volume of glycerol and two volumes of water. They may be then removed, dried and employed in a Soxhlet apparatus for an hour. Thus treated they may be used to advantage with any solvent in the vapour of which water and glycerol are not readily soluble.

Pentosans.—In using the official American method for the estimation of the pentosans in grasses, I am fortunate in having a small supply of phloroglucin, which has been practically unobtainable. Should it not be forthcoming for future determinations some other method must be substituted. That suggested by Jolles (1906) involves the use of oreinol which has also been difficult to procure. Details of a third method which is a modification of the phenylhydrazine method have been given by Menant and Dowell (1919). Brunnich (1907) recommends Tollens' (1902) method for the determination of pentosans, but phloroglucin has, up to the present, been found so satisfactory a reagent that other methods will hardly be adopted as long as it is available.

Moisture.—This has been determined by heating the material to constant weight in the water oven (Brunnich and Smith 1907).

Ash.—In the determination of the ash, the American official method has again been followed.

Carbohydrates, so far, have been determined, as is usually the case, by difference. This method, however, is far from satisfactory, and the writer hopes to be able to estimate them in more detail at a later stage in the work.

v. *Experimental.*

As before mentioned, the experiments described in this paper have been carried out on the air-dried material. Agricultural chemists generally have found that, after allowing for the moisture content, there is no great difference between the chemical constitution of the green and the air-dried material of feeding stuffs (Brunnich and Smith, 1907). Honeamp (1915) carried out numbers of experiments with both, and he considers that, if the material is dried *in vacuo*, there is absolutely no difference in the nutritive value. The analysis of the air-dried material may, perhaps, also be considered the more reliable of the two. The results of the analyses of grasses from various parts of the State are shown in Table I. With regard to the meaning of the terms "crude" protein and "true" protein used in the tables, "crude" protein represents the total nitrogen, as determined by the Kjeldahl Gunning Arnold method before mentioned, multiplied by the factor 6.25. The use of this factor has been subjected to a good deal of criticism in America and elsewhere, but American chemists have not, so far, succeeded in finding any other factor which would give more accurate results in the case of grasses. G. L. Bidwell (1916) writing on this subject, states that, although in the case of wheat and other substances containing a few well known proteins a factor giving better results may be easy to obtain, it is a different matter in the case of substances in which the protein content is of a complex character. He says "consider the labour involved in determining the factor to apply to any one substance. It would be necessary to determine the amount of each protein in that substance then to prepare it in a pure condition so that the percentage of nitrogen might be determined therein. This is a problem for the specialist who has available almost unlimited time and money" (p. 29). He recommends the retention of the factor 6.25 in such cases, and it has therefore been made use of in the preliminary stages of this work.

The term "true" protein is used to designate the precipitate obtained by Stutzer's Reagent. This precipitate is Kjeldahled as in the case of the crude protein, and the result so obtained is multiplied, as before, by the factor 6.25 (Assoen. Offic. Agric. Chemists, 1920). The albuminoid ratio mentioned in the tables is "the ratio of non-nitrogenous to nitrogenous nutrients in any food" (Murray, 1914, p. 325). In order to determine this ratio, the non-nitrogenous nutrients must all be expressed in the terms of one of them—carbohydrates. The amount of ether extract must, therefore, be multiplied by a factor which represents the value of fat as compared with carbohydrates. The factor commonly used is 2.3 (Murray, 1914).

vi. *Discussion of Tables.*

Effect of Soil and Climate on the Chemical Constitution of Grasses.

A good deal of work in this connection has been carried out in the United States (Le Clere and Voder, 1914), notably in Wyoming (Knight, etc., 1908, 1911), where it was found that the percentage of nitrogen appeared to increase with the increase in altitude, while the percentage of crude fibre, under the

TABLE I. *Analyses of Various Grasses, Mostly at First Stage of Growth.*

| Name of Grass and Date Forwarded. | Locality and Nature of Soil. | Stage of Growth. | Total Moisture. (%) | Percentages Air Dried Material. | | | | | | | | |
|--|--|------------------|---------------------|---------------------------------|-------|----------------|---------------|--------------|----------------|-----------|---|----------------------|
| | | | | Moisture. | Ash | Crude Protein. | True Protein. | Crude Fibre. | Ether Extract. | Pentosan. | Carbo-hydrates other than Pentosan. | Albuminoid Ratio. |
| <i>Danthonia semi-annularis</i> Labill. 15 Aug., 1920. (dried in laboratory). | Botanic Gardens. Sandy loam. | 1st | 82.0 | 8.7 | 10.1 | 14.25 | 8.175 | 22.65 | 3.12 | 20.68 | 20.50 | 1 : 3.393 |
| <i>Schedonorus Hookerianus</i> Benth. (Syn. <i>Festuca Hookeriana</i> F.v.M.) 17 Aug., 1920. (dried in laboratory). | Hawkesbury Ag. Coll. Light grey silt. | 1st | 81.0 | 10.10 | 10.8 | 15.56 | 8.065 | 23.50 | 4.82 | 19.19 | 16.05 | 1 : 2.977 |
| <i>S. Hookerianus</i> Benth. 17 Aug., 1920. (dried in laboratory). | Botanic Gardens. Sandy loam. | 1st | 84.58 | 9.08 | 8.27 | 15.75 | 8.39 | 22.90 | 4.448 | 17.73 | 21.822 | 1 : 3.161 |
| <i>S. Hookerianus</i> Benth. 17 Aug., 1920. (dried at Gardens). | Botanic Gardens. Sandy loam. | 1st | 84.59 | 8.99 | 8.276 | 15.75 | 8.41 | 22.89 | 4.42 | 17.692 | 21.982 | 1 : 3.164 |
| <i>D. semi-annularis</i> Labill. 3 Nov., 1920. (dried at Farm). | Cowra State Expt. Farm. Sandy loam, Granite origin. | 1st | 80.89 | 8.89 | 7.13 | 16.48 | 10.85 | 23.30 | 3.53 | 17.03 | 23.64 | 1 : 2.961 |
| <i>S. Hookerianus</i> Benth. 27 Sept., 1920. (dried in laboratory). | Glen Innes Expt. Farm. Gravelly and comparatively poor. | 1st | 79.0 | 9.5 | 8.30 | 15.75 | 6.50 | 22.43 | 3.01 | 17.37 | 23.64 | 1 : 3.044 |
| <i>S. Hookerianus</i> Benth. 3 Oct., 1920. (dried at Farm). | Ditto. | 1st | 85.63 | 9.23 | 8.35 | 16.18 | 11.23 | 22.41 | 3.81 | 16.89 | 23.13 | 1 : 3.015 |
| <i>Panicum prolutum</i> F.v.M. 19 Oct., 1920. (dried at College). | Hawkesbury Ag. Coll. Light grey silt. | 1st | 75.98 | 9.98 | 6.23 | 15.57 | 8.63 | 23.40 | 4.43 | 17.93 | 22.45 | 1 : 3.249 |
| <i>P. prolutum</i> F.v.M.* 8 Nov., 1920. (dried in laboratory). | Ditto. | 2nd | 68.21 | 8.31 | 4.87 | 7.51 | 5.88 | 27.55 | 3.51 | 22.12 | 26.13 | 1 : 7.500 |
| <i>D. semi-annularis</i> Labill. 6 Dec., 1920. (dried in laboratory). | Glen Innes Expt. Farm. Gravelly and comparatively poor. | 3rd | 69.42 | 7.52 | 5.32 | 5.68 | 3.55 | 33.25 | 3.01 | 26.78 | 18.44 | 1 : 9.180 |

* This grass has been cultivated by the Department of Agriculture for the past twelve years under the common name of Coolah Grass.

same conditions, showed a decrease. Also that, in comparing samples collected during the summer with those collected during the winter months, a falling off in the percentage of protein was noticed during the winter months, while the percentage of crude fibre was greater in the winter samples. The percentage of ether extract was found to drop materially in the winter samples.

As may be seen from Table i., *Schedonorus Hookerianus* Benth. (syn. *Festuca Hookeriana* F.v.M.), has been analysed at the first stage before mentioned from Glen Innes, Hawkesbury Agricultural College and the Botanic Gardens. There is apparently very little difference between the protein content of the samples from Hawkesbury and the Botanic Gardens, both of which places are practically at sea level, but that from Glen Innes, at an elevation approximating 4000 feet, shows a distinctly larger amount of protein. As to how much of this variation may be due to difference in altitude, and how much to soil, etc., I am not prepared at this early stage of the work to form any conclusion. At Hawkesbury Agricultural College the soil in which the grasses are grown is a relatively poor one. Dr. Jensen (1912) describes it as a light grey silt, strongly acid in character. The experimentalist at Glen Innes states that the soil of the grass plots there is generally regarded as poor—though suitable for cereal growing—and decidedly clayey, light in colour and inclined to be gravelly. It is apparently derived from a basalt, probably bauxitic in character.

Again in comparing the analyses of *Danthonia semi-annularis* Labill. in the first stage from the State Experimental Farm at Cowra, which is at an altitude of about 1000 feet, with a sample from the Botanic Gardens in the same stage, the protein content of the Cowra Sample was found to be higher than that from the Botanic Gardens. Jensen (1914), writing on "The Soils of Cowra" states that the dominant soil there is a warm red loam, overlying a stony and fairly heavy clay subsoil, which merges into rotten granite. He also says "we get granite dominating on the Experiment Farms of Cowra and Bathurst." The Botanic Gardens soil is described as a sandy loam, and is decidedly poor, according to Mr. Ward, Superintendent at the Gardens. It will be interesting to see whether further work on samples from localities differing in soil, altitude, etc., will tend to confirm these particulars. This is, I think, an important phase of the question.

So far there are no noticeable variations between summer and winter samples, but these may become apparent when a greater number of grasses have been examined. In connection with the results given in Table i., a somewhat remarkable variation is seen in two samples of *Schedonorus Hookerianus*, obtained from Glen Innes State Experiment Farm. One of these samples was sent on to me as soon as cut, and, owing to some delay in transit, arrived in a decidedly wilted condition. It was air-dried in the laboratory. The other sample was dried at the Farm under natural conditions. The first sample was found to contain 0.43% less of crude protein than the second. When, however, the true protein nitrogen, or albuminoid nitrogen, as it is called in America, was estimated in each, the wilted sample was found to contain 5.73% less of the so-called true protein than the sample which was dried on the Farm. The percentage of amino acid, amides, etc., was, however, correspondingly larger, as these substances represent the difference between the so-called crude and true protein. The actual figures are:—

| | Crude Protein. | True Protein (albuminoid N). |
|--|----------------|------------------------------|
| <i>Schedonorus Hookerianus</i> , first stage (wilted sample) | 15.75% | .. 6.50% |
| <i>Schedonorus Hookerianus</i> , first stage (good sample) | 16.18% | .. 11.23% |

TABLE II.

Showing variation in Protein Content at different Stages of Growth.

| Name and Date. | Locality and Nature of Soil. | Stage of Growth. | % Crude Protein. | % True Protein. | Remarks. |
|---|---|------------------|------------------|-----------------|------------------------------|
| <i>Schedonorus Hookerianus</i> 27 Sept., 1920. (dried in laboratory). | Glen Innes Experimental Farm (4000 feet above sea level). Gravelly and comparatively poor. | 1st | 15.75 | 6.50 | |
| <i>S. Hookerianus</i> . 3 Oct., 1920. (dried at Farm). | Ditto. | 1st | 16.18 | 11.23 | |
| <i>S. Hookerianus</i> . 9 Feb., 1921. (dried at Farm). | Ditto. | 2nd | 10.706 | 7.895 | Flowers not long fertilised. |
| <i>Panicum prolatum</i> . 19 Oct., 1920. (dried at College). | Hawkesbury Agrie. College. Light grey silt. | 1st | 15.57 | 8.63 | |
| <i>P. prolatum</i> . 8 Nov., 1920. (dried at College). | Ditto. | 2nd | 7.51 | 5.88 | Flowers not long fertilised. |
| <i>P. prolatum</i> . 12 Dec., 1920. (dried at College). | Ditto. | 3rd | 6.74 | 5.10 | Seed set. |
| <i>Eragrostis leptostachya</i> . 12 Jan., 1921. (dried at College). | Ditto. | 2nd | 11.98 | 9.20 | Seed just beginning to set. |
| <i>E. leptostachya</i> . 12 Jan., 1921. (dried at College). | Ditto. | 3rd | 8.137 | 7.20 | Seed set. |
| <i>S. Hookerianus</i> . 15 Aug., 1920. (dried in laboratory). | Botanic Gardens. Sandy loam. | 1st | 15.75 | 8.39 | |
| <i>S. Hookerianus</i> . 15 Aug., 1920. (dried at Gardens). | Ditto. | 1st | 15.75 | 8.41 | |
| <i>S. Hookerianus</i> . 24 Jan., 1921. (dried at Gardens). | Ditto. | 2nd | 10.325 | 8.025 | Seed beginning to set. |
| <i>Danthonia semi-annularis</i> . 15 Aug., 1920. (dried in laboratory). | Ditto. | 1st | 14.25 | 8.175 | |
| <i>S. Hookerianus</i> . 17 Aug., 1920. (dried in laboratory). | Hawkesbury Agrie. College. Light grey silt. | 1st | 15.56 | 8.065 | |
| <i>D. semi-annularis</i> . 3 Nov., 1920. (dried at Farm). | Cowra Experimental Farm (978 feet above sea level). Sandy loam, granite origin. | 1st | 16.48 | 10.85 | |
| <i>D. semi-annularis</i> . 6 Dec., 1920. (dried in laboratory). | Glen Innes Experimental Farm. (4000 feet above sea level). Gravelly and comparatively poor. | 3rd | 5.68 | 3.557 | Seed set. |
| <i>Danthonia pilosa</i> (?) R.Br.* 29 Dec., 1920. (dried on Farm). | Yanco Experimental Farm. Chocolate loam, with hard clayey subsoil. | 2nd | 8.05 | 6.50 | Seed beginning to set. |
| <i>Panicum prolatum</i> . 24 Jan., 1921. (dried at Gardens). | Botanic Gardens. Sandy loam. | 3rd | 5.952 | 4.230 | Seed set. |
| <i>Panicum decompositum</i> R.Br. 24 Jan., 1921. (dried at Gardens). | Ditto. | 3rd | 5.325 | 4.20 | Seed set. |
| <i>Pollinia fulva</i> Benth. 24 Jan., 1921. (dried in laboratory). | Ditto. | 2nd | 9.320 | 7.152 | Seed beginning to set. |

* The genus *Danthonia* is now under revision by Messrs. Cheel and Breakwell, of the Botanic Gardens staff, and it is quite possible that the *D. pilosa* group will be split up and renamed.

as will be seen from Table i. Analyses carried out on other grasses at this stage show that the non-protein nitrogen is usually about 40% of the total nitrogen, so that, in the case of the wilted sample, there has been apparently, a breaking down of the proteins into their cleavage products. I hope to carry out further experiments under similar conditions, in order to verify the result which has been shown above. The non-proteins are found to be especially abundant in immature plants, where the protein formation has not yet been completed, and in fermented foods such as silage, where the proteins have been partially decomposed (Sleeter Bull, 1916). Some such decomposition has apparently taken place in the wilted sample of grass from Glen Innes. Sleeter Bull (1916) also considers that there are considerable differences in the nutritive value of the amino acids, some being essential to life, while others are essential to growth only. "Therefore," he says, "different proteins may differ considerably in nutritive value," e.g., zein, the principal protein of maize, has no lysine or tryptophane (Osborne and Mendel, 1916). So far no great variations have been observed in the percentages of ether extract. The crude fibre content appears to be rather low compared with American grasses (Griffiths, etc., 1915), the pentosan also showing a slightly lower percentage. If these results are borne out by subsequent investigation, our native grasses should compare favourably with those of the United States, as the percentage of protein appears to be about the same in each. It will be noticed that the percentage of ash shows a decided drop at the second stage of growth. There is, of course, nothing unusual about this. Preston (1887) and Knight, Hepner and Nelson (1911), stress the importance of giving particulars as to locality, soil, etc., in these analyses. They have therefore been included in Table i.

On examination of samples at the different stages as given in Table ii., the highest crude protein content, so far, has been found to occur in samples collected at the first stage and to diminish gradually as the grass grows older. As before mentioned, however, crude protein at this stage is considered to consist largely of amino acids, amides, etc. (Sleeter Bull, 1916), which although highly important constituents (Osborne and Mendel, 1916; Nollan, 1915), have hardly such a high nutritive value as the true proteins.

In grasses examined at the second stage the protein content apparently lessens considerably (Söderbaum, 1918). I have examined the flowers of some grasses at this stage, and results are given in Table iii. I find that, at this stage, apparently a considerable proportion of the protein is found in the flower itself, and that considerably less occurs in the leaves and stems than was the case at an earlier stage. At the third stage still less appears to occur in the leaves and more in the seed (Petrie, 1911; Schulze and Schultz, 1909).

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TABLE III.

Showing Amount of Protein in Flowers.

| Name. | Locality and Date. | Percentage of Crude Protein in Flowers. | Remarks. |
|---|---|---|--------------------------------|
| <i>Panicum prolutum</i> (dried in laboratory). | Hawkesbury Agric. College. 10 Nov., 1921. | 13.823 | Not long fertilised. |
| <i>Schedonorus Hookerianus</i> . (dried at Farm). | Glen Innes. 9 Feb., 1921. | 13.925 | Not long fertilised. |
| <i>Eragrostis leptostachya</i> . (dried at College). | Hawkesbury Agric. College. 12 Jan., 1921. | 14.735 | Seed is just beginning to set. |
| <i>Danthonia semi-annularis</i> . (dried at Farm). | Glen Innes. 9 Feb., 1921. | 12.302 | Unfertilised. |
| <i>Schedonorus Hookerianus</i> . (dried at Gardens). | Botanic Gardens. 24 Jan., 1921. | 14.023 | Seed is just beginning to set. |

The grasses mentioned in this table are the only ones which have been procurable so far, in the flowering stage.

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